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Light-Limitation on Predator-Prey Interactions: Consequences for Metabolism and Locomotion of Deep-Sea Cephalopods

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Abstract. The present study attempts to correlate the metabolism and locomotory behavior of 25 species of midwater Cephalopoda from California and Hawaii with the maximal activities of key metabolic enzymes in various locomotory muscle tissues. Citrate synthase (CS) and octopine dehydrogenase (ODH) activities were used as indicators of aerobic and anaerobic metabolic potential respectively. CS activity in mantle muscle is highly correlated with whole-animal rates of oxygen consumption, whereas ODH activity in mantle muscle is significantly correlated with a species' ability to buffer the acidic end-products of anaerobic metabolism. Both CS and ODH activities in mantle muscle declined strongly with a species' habitat depth. For example, CS and ODH activities ranged respectively from 0.04 units g^{-1} and 0.03 units g^{-1} in the deep-living squid *Joubiniteuthis portieri*, to 8.13 units g^{-1} and 420 units g^{-1} in the epipelagic squid *Sthenoteuthis oualaniensis*. The relationships between enzymatic activities and depth are consistent with similar patterns observed for whole-animal oxygen consumption. This pattern is believed to result from a relaxation, among deep-living species, in the need for strong locomotory abilities for visual predator/prey interactions; the relaxation is due to light-limitation in the deep sea. Intraspecific scaling patterns for ODH activities may, for species that migrate ontogenetically to great depths, reflect the counteracting effects of body size and light on predator-prey detection distance. When scaled allometrically, enzymatic activities for the giant squid, *Architeuthis* sp., suggest a fairly active aerobic metabolism but little burst swimming capacity. Interspecific differences in the relative distributions of enzymatic activities in fin, mantle, and arm tissue suggest an increased reliance on fin and arm muscle for locomotion among deep-living species. We suggest that, where high-speed locomotion is not required, more efficient means of locomotion, such as fin swimming or medusoid arm propulsion, are more prevalent.

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Introduction

The evolutionary path leading to modern cephalopods is poorly known due to a limited fossil record of soft-bodied cephalopods. There has, however, been much speculation based on the better fossil record of the shelled cephalopods from which modern forms evolved. Packard (1972) noted that most shelled cephalopods went extinct and modern forms, the coleoids, radiated over roughly the same geological time period as did the fishes. He further hypothesized that "competition" (which in his definition consisted of all "negative" biotic interactions, including predation) with these fishes through visually based interactions led to the (convergent) evolution of modern coleoid cephalopods (Packard, 1972). Although the timing of Packard's scenario is now in doubt, the influence of visually mediated "competition" with vertebrate predators on cephalopod evolution is widely accepted (Aronson, 1991). Packard's model provides a useful framework within which to address physiological and behavioral adaptations to the deep sea.

Packard's evolutionary model suggests that cephalopods initially retreated into the deep sea to avoid vertebrate predators encroaching out into oceanic waters. A suite of

adaptations for mobility allowed them to compete effectively with fishes in visual predator/prey interactions and facilitated the reinvasion of shallow waters by modern coleoid cephalopods (Packard, 1972).

Among these adaptations was the reduction and internalization of the shell and later, an increase in mantle volume and the development of powerful propulsive mantle muscles (Wells and O'Dor, 1991). As a result, some squids today are among the most powerful swimmers in the oceans (O'Dor and Shadwick, 1989). Squids that occupy the well-lit epipelagic waters have metabolic rates unmatched by any other aquatic poikilotherm. Epipelagic squids, most of which are negatively buoyant, rely somewhat on fins for hovering, maneuvering, and stabilization (Hoar *et al.*, 1994). When attacked, however, they escape using powerful jet propulsion that produces considerable speed and may even thrust the animals out of the water (Cole and Gilbert, 1970). Both fishes and cephalopods move forward by accelerating water backwards. Fish undulate through the water, pushing large volumes back at low speeds with their broad tails. Jetting squids must force the small volumes of water taken into the mantle cavity out through an even smaller funnel opening (O'Dor and Webber, 1986). Squids must accelerate this water to much higher speeds to gain similar momentum. This results in low Froude efficiency (Vogel, 1994). A squid, therefore, takes about twice the energy to go only half as fast as the average fish (O'Dor and Webber, 1986). This constraint is compensated for by high metabolic rates, remarkable circulatory systems, and upregulated biochemical pathways (O'Dor and Webber, 1986).

Those species that have either remained in or reinvaded the deep sea do not interact with fishes or other taxa in extended visually based interactions. Deep-sea fishes and cephalopods do possess well-developed eyes and do use bioluminescent displays to attract prey or confuse predators. However, bioluminescence is a relatively weak, transient light source. Interactions under such light regimes are likely to take place over short distances that do not require strong locomotory abilities (Marshall, 1979; Herring *et al.*, 1994; Fleisher and Case, 1995). Life without extended, visually based "competition" should be accompanied by a reduction in locomotory capacity, and therefore, energy usage or metabolic rate. This is the basis of the visual interactions hypothesis put forward by Childress and colleagues (see Childress, 1995, for review). The visual interactions hypothesis states that reduced metabolic rates among deep-living fishes and crustaceans relative to shallower living species reflects reduced locomotory requirements for visually cued predator/prey interactions in the low levels of ambient light found in the deep sea. In support of this hypothesis, Seibel *et al.* (1997) reported a strong decline in oxygen consumption rate with increasing habitat depth among pelagic cephalopods.

Seibel *et al.* (1997) further observed that the decline in metabolic rates with increasing habitat depth among pelagic cephalopods is more pronounced than that reported for fishes or crustaceans (Childress, 1995). Because fin swimming is inherently more efficient than jet propulsion, some of this reduction in energy usage in deep-living cephalopods could result from the use of fins, instead of jet propulsion, as the primary means of locomotion. Where high speeds are not a priority, dependence on fins for locomotion reduces the cost of transport. Direct observations of the behavior and locomotion of deep-living pelagic animals are limited to occasional sightings from submersibles and remotely operated vehicles (Vecchione and Roper, 1991; Hunt, 1996; Roper and Vecchione, 1997; Villanueva *et al.*, 1997; Vecchione and Young, 1997; Hunt and Seibel, 2000). These observations, while extremely important, can give only qualitative information on the locomotory abilities of these organisms.

One method for quantifying the metabolic and locomotory capacities of animals is to determine the maximum activities of enzymes unique to different catabolic pathways. Enzymatic activities in various locomotory muscle tissues have been shown to correlate well with locomotory behavior in cephalopods (Baldwin and England, 1980; Baldwin, 1982; Seibel *et al.*, 1998; Hunt and Seibel, 2000). The present study measured the enzymatic activities variously in fin, mantle, arm, and heart muscle of pelagic cephalopods to investigate the interplay between metabolism and locomotion, especially as these parameters vary with habitat depth.

Materials and Methods

Collection

Cephalopods were captured on nine cruises aboard the R/V *Point Sur* and R/V *New Horizon* between September 1992 and September 1996. Sampling was primarily in an area 160 km west of Point Conception, California (34°37′N, 122°42′W to 34°30′N, 123°20′W), and off Oahu, Hawaii (21°20′N, 158°20′W to 21°35′N, 158°35′W). Animals were collected in an opening/closing Mother Tucker trawl with a 10-m^2 mouth. The net was equipped with a 30-l thermally protecting cod end that reduced mechanical damage and heat shock to animals during recovery (Childress *et al.*, 1978). Ship speed was kept very low (0.5–1 kn) to decrease turbulence and abrasion in the net and to reduce the number of animals collected in the cod end. Upon reaching the surface, specimens were weighed on a precision, motion-compensated shipboard balance (Childress and Mickel, 1980) and immediately dissected. Tissues were frozen in liquid nitrogen. All animals used in this study were alive on board ship prior to dissection, with the exception of the giant squid, *Architeuthis* sp. (see below). Animals were identified to species with the aid of several sources (Young, 1972; Roper *et al.*, 1984; Sweeney *et al.*, 1992; F. G.

Hochberg, pers. comm.; R. E. Young, pers. comm.; N. A. Voss, pers. comm.). Voucher specimens were preserved for verification of identifications and are deposited at the Museum of Natural History, Santa Barbara, California.

A single specimen of the giant squid, *Architeuthis* sp., was collected dead on 3 June 1999 by the charterboat *Top Dog* (Capt. Phil Slaga and Mate Bo Spieler) in the Gulf Stream off the Florida Keys and brought to the Rosenstiel School of Marine and Atmospheric Science (RSMAS). The specimen was in "fresh" condition but missing the distal one-third of the mantle (fins), the tentacles, and the ends of all arms but R1. The specimen was kept either on ice or in a refrigerated cold room for about 3 days prior to dissection. The mantle length (ML) was approximately 1000 mm (700 mm + est 300 mm). Using the relationship described by O'Dor (1988; Mass = 20 ML[m]^3) for ommastrephid squids including the jumbo squid, *Dosidicus gigas*, we predict a wet weight of approximately 20 kg for the present specimen. The mass–length relationship described by O'Dor (1988) appears consistent with mass–length values reported for *Architeuthis* in the literature (see Ellis, 1998, for a comprehensive list of collected specimens). The specimen of *Architeuthis* sp. (UMML31.312b) is housed in the Marine Invertebrate Museum at RSMAS.

Dissections

Tissue was extracted variously from the left ventral mantle, left fin, left second arm, and left branchial heart. It has been shown that mantle muscle of some cephalopods is divided into distinct layers analogous to vertebrate white and red muscle (Bone *et al.*, 1981; Mommsen *et al.*, 1981). No attempt was made to locate or sample these layers separately. Although the relative abundance of "red" or "white" muscle fiber types in differently sized individuals may be a proximal cause for some of the scaling patterns observed (Preuss *et al.*, 1997), it cannot be an evolutionary driving force for the scaling relationships (Seibel *et al.*, 1998). Some deep-living specimens have a gelatinous tissue covering the musculature. We attempted to remove this tissue from the muscle prior to freezing.

Enzymes

Sections of approximately 0.5 g of fin, mantle or arm, or whole branchial hearts were homogenized in varying dilutions of 0.01 *M* Tris homogenization buffer, pH 7.5, at 10°C in Duall hand-held glass homogenizers kept on ice. Homogenate was centrifuged at 6600 × *g* for 5 min at 5°C. Aliquots of 25–50 μl of sample supernatant were placed in 1-ml quartz cuvettes under nonlimiting substrate conditions to be assayed for enzyme activity. Maximal enzymatic activities (V_{max}) were measured with a Shimadzu UV160U spectrophotometer equipped with a water-jacketed cuvette holder connected to a recirculating water bath. Measurements were carried out at 20°C and atmospheric pressure.

Octopine dehydrogenase (ODH; EC.1.5.1.11) and lactate dehydrogenase (LDH; EC.1.1.1.8) were assayed as indicators of anaerobic metabolic potential according to the methods of Baldwin and England (1980). ODH catalyzes the terminal glycolytic reaction in cephalopods, resulting in the reductive condensation of pyruvate and arginine to form octopine. This reaction maintains redox balance in the cell when oxygen is limiting. Because the rate of this reaction is the sum of ODH and LDH activities, ODH values were obtained by subtracting the rate due to LDH assayed in the absence of arginine. Citrate synthase (CS; EC.4.1.3.7) was used as a correlate of aerobic metabolism. It was assayed according to the methods of Thuesen and Childress (1994). CS is an important regulatory enzyme and functions in the first step of the citric acid cycle (Hochachka *et al.*, 1975). Species' mean enzymatic activities were plotted as a function of minimum depth of occurrence (MDO), defined as the depth below which 90% of the individuals of a given species were captured, and of body mass.

Statistical analysis

All data analyses were performed with Statview ver. 2.0 or SuperANOVA ver. 1.11 statistics programs (Abacus Concepts, Inc., Berkeley, CA). Simple linear regressions, *t* tests and analysis of covariance (ANCOVA) were used. Mean values given are followed by the standard error. Confidence intervals for regression exponents are at the 95% level, *P* values for regression coefficients are from *F* tests. ANCOVA was used to test whether the slopes and elevations of the various relationships were significantly different from zero and from each other. Regression slopes were declared significant when their slopes differed from zero at the 95% confidence level. In some cases nonparametric tests were used (Kendall rank correlation coefficient).

Results

The activities of octopine dehydrogenase (ODH), lactate dehydrogenase (LDH), and citrate synthase (CS) in mantle, fin, arm, and branchial heart muscle tissue for 26 species of pelagic cephalopods are presented in Table 1. Mean ODH activities of mantle muscle are significantly correlated with the buffering capacity of mantle tissue (Seibel *et al.*, 1997; $y = 1.11x + 11.75$; $r^2 = 0.81$; $P = 0.0025$; $n = 8$; Fig. 1). This result supports the use of this enzyme as an indicator of anaerobic metabolic potential. With the exception of mantle muscle for the Bolitaenidae (*Japetella* and *Eledonella*) and branchial heart tissue in all species, LDH activity was generally less than 10% of ODH activity. Mean CS activities are significantly correlated with mean oxygen consumption rates (Seibel *et al.*, 1997; $y = 0.72x - 0.04$; $r^2 = 0.85$;

Table 1

Enzymatic activity expressed as units (μmoles substrate converted to product min^{-1}) g^{-1} wet weight in mantle (m), fin (f), arm (a) and heart (h) tissue of pelagic cephalopods

Species	MDO (m)	tissue	*n*	Wet weight (g) range	Enzymatic activity (mean ± SE)* CS (units g^{-1})	ODH (units g^{-1})	LDH (units g^{-1})
California							
Order Teuthoidea							
Gonatus onyx	100	m	4	1.06-31.08	7.85 ± 1.47	29.82 ± 7.26	1.17 ± 0.15
Brooding specimens		m	2	99.68-138.6	0.88, 1.63	1.61, 12.47	0.03, 0.51
		a	1		1.65	23.34	1.61
Gonatus pyros	100	m	1	6.12	7.72	28.14	1.25
		f	1		1.50	9.71	1.23
		a	1		0.84	5.92	0.75
		h	1		13.82	18.01	10.29
Abraliopsis falco	50	m	5	0.51–8.16	6.27 ± 0.63	40.96 ± 5.46	1.83 ± 0.79
		f	4	0.51–8.16	7.80 ± 0.42	25.69 ± 6.53	1.69 ± 0.19
		a	1	0.51	2.89	10.19	2.43
Pterygioteuthis gemmata	100	m	2	0.13, 0.43	5.47, 9.87	42.76, 77.65	1.66, 3.80
Histioteuthis heteropsis	150	m	11	0.47–33.97	1.68 ± 0.21	11.40 ± 0.80	0.45 ± 0.10
		f	5	1.79–12.68	1.46 ± 0.36	10.34 ± 4.09	0.47 ± 0.18
		a	9	0.47–11.41	0.59 ± 0.15	0.89 ± 0.15	0.18 ± 0.05
		h	3	11.41–33.97	5.73 ± 2.34	2.23 ± 0.09	n.d.
Octopoteuthis deletron	100	m	1	8.19	0.10	0.06	0.07
		f	1		1.03	9.84	0.26
		a	1		0.21	2.97	0.17
Chiroteuthis calyx	300	m	7	5.93–75.86	1.37 ± 0.26	50.69 ± 8.99	0.68 ± 0.09
		f	5	5.93–41.45	5.49 ± 0.59	69.02 ± 10.17	1.33 ± 0.41
		a	5	5.93–89.02	0.11 ± 0.05	1.83 ± 0.81	0.10 ± 0.05
		h	4	5.93–89.02	7.57 ± 2.89	16.19 ± 8.27	5.41 ± 1.41
Galiteuthis phyllura	300	m	3	5.19–106.0	1.31 ± 0.66	50.15 ± 15.77	0.41 ± 0.04
		f	3		2.05 ± 1.33	30.37 ± 8.70	0.98 ± 0.25
		a	3		1.77 ± 0.37	31.48 ± 1.34	0.84 ± 0.19
Cranchia scabra	10	m	1	35.39	1.41	29.39	1.45
		f	1		2.66	27.85	0.92
		a	1		1.06	23.15	1.61
Bathyteuthis berryi	800	m	2	1.7, 24.9	2.51, 0.89	0.27, 1.85	0.06, 0.08
		f	2		0.45, 1.73	0.43, 1.37	0.15, 0.18
		a	2		0.59, 1.58	0.63, 1.78	0.09, 0.43
Order Octopoda							
Japetella heathi	600	m	5	9.75–162.5	0.19 ± 0.03	0.32 ± 0.21	3.06 ± 0.65
		a	3	9.75–91.59	1.68 ± 0.86	22.27 ± 10.40	4.54 ± 1.63
Order Vampyromorpha							
Vampyroteuthis infernalis	600	m	25	0.45–1050	0.21 ± 0.02	3.07 ± 0.48	0.11 ± 0.02
		f	23	4.74–1050	0.52 ± 0.07	5.37 ± 1.22	0.17 ± 0.03
		a	14	0.45–666	0.08 ± 0.01	1.34 ± 0.24	0.02 ± 0.01
Florida							
Order Teuthoidea							
Architeuthis sp.	—	m	1	2×10^5	0.76	7.22	0.51
Hawaii							
Order Sepioidea							
Heteroteuthis hawaiiensis	110	m	1	5.06	4.91	81.17	1.65
		f	1		3.20	47.64	0.86
Order Teuthoidea							
Sthenoteuthis oualaniensis	10	m	1	110.0	8.13	420.9	1.34
		f	1		2.28	133.04	1.75
		a	1		1.96	25.33	2.19
		h	1		26.66	26.69	9.41
Brachioteuthis sp.	10	m	1	1.49	4.74	86.79	1.17
		f	1		8.64	29.79	1.12
		a	1		1.51	10.36	1.02

Table 1 (*Continued*)

Species	MDO (m)	tissue	*n*	Wet weight (g) range	Enzymatic activity (mean ± SE)* CS (units g^{-1})	ODH (units g^{-1})	LDH (units g^{-1})
Leachia pacifica	50	m	1	0.51	0.50	18.52	1.96
		f	1		3.63	78.08	2.76
		a	1		3.06	30.90	7.86
Abraliopsis pacificus	50	m	2	0.37,1.30	3.58, 6.81	61.33, 113.08	1.33, 1.41
		f	1	1.30	10.25	28.37	0.98
		a	2	0.37,1.30	0.54, 1.48	44.73, 49.87	0.84, 0.76
Histioteuthis hoylei	150	m	3	1.27–5.19	2.86 ± 0.71	0.74 ± 0.13	0.60 ± 0.05
		f	3		5.82 ± 1.59	3.56 ± 1.98	0.14 ± 0.02
		a	3		0.34 ± 0.21	0.09 ± 0.06	0.10 ± 0.02
Mastigoteuthis famelica	375	m	2	4.06,7.32	0.61, 0.85	6.22, 7.45	0.06, 0.26
		f	2		1.26, 2.09	2.66, 3.85	0.08, 0.16
		a	2		0.26, 0.39	1.61, 2.44	0.08, 0.24
		h	1	7.32	7.22	4.96	1.03
Chiroteuthis imperator	300	m	4	14.94–53.36	0.26 ± 0.24	30.90 ± 5.31	0.68 ± 0.09
		f	3	27.65–53.36	5.33 ± 1.03	44.49 ± 10.27	1.33 ± 0.41
		a	3		0.25 ± 0.05	4.16 ± 1.80	0.10 ± 0.05
		h	2		17.98, 22.47	15.64, 31.26	7.70, 7.72
Joubiniteuthis portieri	500	m	2	36.00,48.99	0.01, 0.06	0.04, 0.02	n.d.
		f	2		5.33, 3.84	0.05, 0.05	0.00, 0.06
		a	2		0.02, 0.02	0.01, 0.06	0.02, 0.00
		h	2		0.08, 0.86	0.04, 0.52	0.43, 0.51
Liocranchia valdivia	500	m	6	0.92–21.28	1.64 ± 0.43	28.08 ± 4.37	0.46 ± 0.23
		a	4	1.49–2.47	2.25 ± 0.62	14.00 ± 2.05	0.91 ± 0.28
Sandilops sp.	300	m	2	1.22,6.44	1.22, 1.80	17.03, 17.20	0.06, 0.29
		f	2		0.78, 1.96	6.07, 14.83	0.12, 0.15
		a	1	1.22	2.89	17.69	0.32
Taonius pavo	400	m	1	1.06	3.16	22.87	0.52
Order Octopoda							
Japetella diaphana	700	m	11	8.96–325.7	0.16 ± 0.02	0.39 ± 0.14	2.59 ± 0.30
		a	4		0.44 ± 0.11	17.24 ± 5.62	2.36 ± 0.61
		h	3		2.39 ± 0.97	n.d.	7.50 ± 2.74
Eledonella pygmaea	975	m	4	27.65–300.0	0.25 ± 0.03	1.19 ± 0.42	1.74 ± 0.30
		a	4		0.26 ± 0.07	9.55 ± 2.51	0.78 ± 0.21
		h	3		1.61 ± 0.64	0.06 ± 0.06	2.78 ± 0.06

Measurements were made at 20 C. Minimum depths of adult occurrence (MDO) are also listed.

* CS: citrate synthase; ODH: octopine dehydrogenase; LDH: lactate dehydrogenase; n.d. not detected; units, μmole substrate converted to product per min.

$P = 0.0001$; $n = 21$), which supports the use of CS as an indicator of aerobic metabolic potential (Fig. 2).

The highest CS activities overall were found in branchial heart muscle; mean values ranged from 0.47 units g^{-1} in the deep-living squid *Joubiniteuthis portieri*, to 26.66 units g^{-1} in the epipelagic squid *Sthenoteuthis oualaniensis*. Mean citrate synthase activities of mantle muscle ranged from 0.04 units g^{-1} for *J. portieri* to 8.13 units g^{-1} for *S. oualaniensis*. For comparison, CS activity in mantle muscle of the market squid, *Loligo opalescens*, is 8.0 (Ballantyne *et al.*, 1981). The highest CS activity in fin muscle was found in *Abraliopsis pacificus* (10.25 units g^{-1}). The lowest fin CS activity was found in *Octopoteuthis deletron* (1.03 units g^{-1}).

Mean ODH activity in mantle muscle ranged from 0.03 units g^{-1} for *Joubiniteuthis portieri* to 420.9 units g^{-1} in *Sthenoteuthis oualaniensis*. For comparison, ODH activities in *Illex illecebrosus*, another active ommastrephid squid, ranged from 297 units g^{-1} in outer mantle muscle to 860 units g^{-1} in middle mantle muscle (Mommsen *et al.*, 1981). Mean ODH activity in fin muscle ranged from 0.05 units g^{-1} in *J. portieri* to 133.04 units g^{-1} in *S. oualaniensis*.

Joubiniteuthis portieri is unusual in having extremely low activities of both CS and ODH in mantle muscle. ODH was also virtually absent from fin muscle of this species. Fin CS activity, however, was similar to that found in related squid families. The family Bolitaenidae (*Japetella* and *Eledonella*) differed from others in having higher activities of LDH than of ODH in mantle muscle. However, ODH in arm tissue was much higher than LDH in arm or mantle tissue. Except in the octopods and cranchiid squids, CS activity in

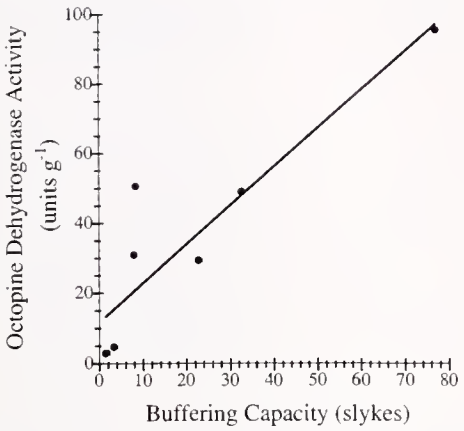

Figure 1. Correlation between octopine dehydrogenase activity (units g^{-1}) and buffering capacity (slykes) of pelagic cephalopods (Seibel *et al.*, 1997). The regression is significant ($y = 1.11x + 11.75$; $r^2 = 0.81$; $P = 0.0025$; $n = 8$).

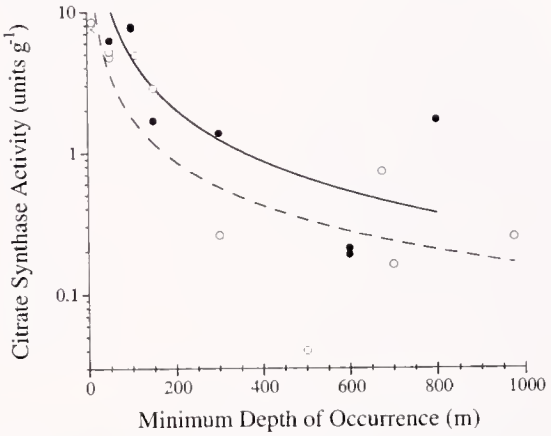

Figure 3. Mean citrate synthase activity in mantle muscle declines significantly with increasing minimum depth of occurrence (m) in Californian (solid circles, solid line; $y = 1398.8x^{-1.23\pm0.32}$; $r^2 = 0.68$; $P = 0.01$; $n = 9$) and Hawaiian (open circles, dashed line; $y = 227.5x^{-1.06\pm0.57}$; $r^2 = 0.69$; $P = 0.0029$; $n = 10$) pelagic cephalopods. For comparison, mantle muscle CS activity for *Loligo opalescens* is also shown (×; 8.0 units g^{-1}; Ballantyne *et al.*, 1981).

the arm tissue was generally lower than that found in either mantle or fin tissue.

Depth

There was a strong decline in mean mantle muscle CS activity with increasing minimum depth of occurrence (Fig. 3) in species from both California ($y = 1398.8x^{-1.23\pm0.32}$; $r^2 = 0.68$; $P = 0.01$; $n = 9$) and Hawaii ($y = 227.5x^{-1.06\pm0.57}$; $r^2 = 0.69$; $P = 0.0029$; $n = 10$). There was also a strong decline in mean ODH activity with increasing MDO (Fig. 4) in species from both California ($y = 3.78 \times 10^4 x^{-1.54\pm0.41}$; $r^2 = 0.67$; $P = 0.007$; $n = 9$) and Hawaii ($y = 2.88 \times 10^4 x^{-1.60\pm1.11}$; $r^2 = 0.58$; $P = 0.01$; $n = 10$). There were not significant relationships between CS or ODH and MDO in fin or arm tissue for either California or Hawaii ($P > 0.05$). Included in these analyses were *Vampyroteuthis infernalis* (Seibel *et al.*, 1998) and *Gonatus onyx* (Hunt and Seibel, 2000). Citrate synthase ($y = 192.87x^{-0.66\pm0.63}$; $r^2 = 0.47$; $P = 0.04$; $n = 9$) and ODH (Kendall rank correlation coefficient; $P = 0.02$) activity in branchial heart tissue also declined significantly with in-

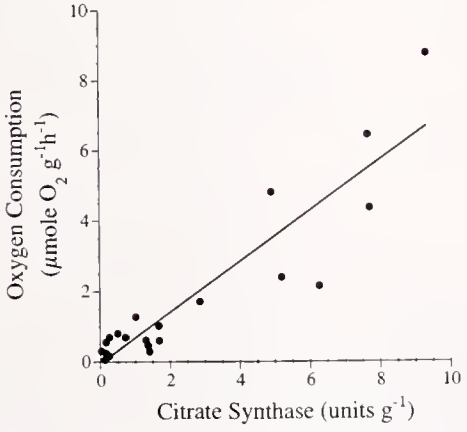

Figure 2. Correlation between mean citrate synthase activity (units g^{-1}) and mean mass-specific oxygen consumption rate (μmole O_2 $g^{-1}h^{-1}$; Seibel *et al.*, 1997) for pelagic cephalopods. The regression is significant ($y = -0.043 + 0.72x$; $r^2 = 0.85$; $P = 0.0001$; $n = 21$).

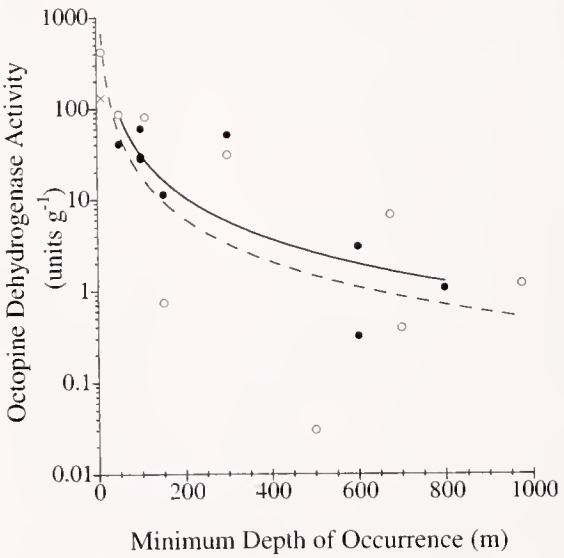

Figure 4. Mean octopine dehydrogenase activity in mantle muscle also declined significantly with increasing MDO (m) in Californian ($y = 3.78 \times 10^4 x^{-1.54\pm0.41}$; $r^2 = 0.67$; $P = 0.007$; $n = 9$) and Hawaiian ($y = 2.88 \times 10^4 x^{-1.60\pm1.11}$; $r^2 = 0.58$; $P = 0.01$; $n = 10$) pelagic cephalopods. Symbols as in Fig. 3. For comparison, mantle muscle ODH activity for *Loligo opalescens* is also shown (×; 132 units g^{-1}; Ballantyne *et al.*, 1981).

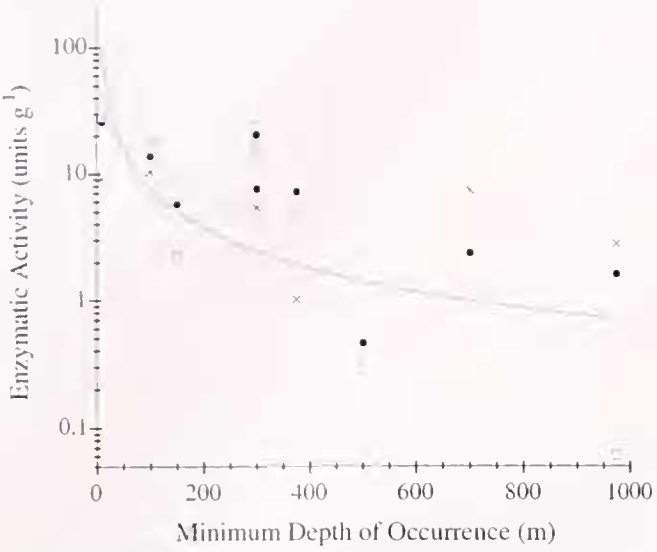

Figure 5. Mean activities of citrate synthase ($y = 192.87x^{-0.66\pm0.63}$; $r^2 = 0.47$; $P = 0.04$; $n = 9$), octopine dehydrogenase ($y = 845x^{-1.10\pm0.50}$; $r^2 = 0.38$; Kendall rank correlation coefficient, $P = 0.02$), and lactate dehydrogenase (×) in branchial heart of pelagic cephalopods as a function of minimum depth of occurrence. Symbols as in Fig. 3.

creasing MDO (Fig. 5). There was no significant relationship between heart LDH activity and MDO. There were no differences between Hawaiian and Californian animals for any parameter measured (ANCOVA; $P > 0.05$).

Excluded from the analyses with depth were species of the family Cranchiidae and *Octopoteuthis deletron*. The use of transparency for crypsis by cranchiid squids relieves them of some of the selective pressure associated with the well-lit epipelagic zone (Seapy and Young, 1986; Seibel *et al.*, 1997; Johnsen and Widder, 1998). In fact, some cranchiid species remain in near-surface waters until adulthood and then migrate suddenly to great depths where spawning occurs (Young, 1975). On the basis of the depths provided in Table 1 (estimated from Roper and Young, 1975), there is no relationship between any enzymatic activities and MDO for cranchiid squids. *O. deletron* is unique in that the fins comprise a larger percentage of the body weight than does mantle muscle (B. A. Seibel, unpubl. data). Therefore, the enzymatic activities of mantle muscle may not provide accurate estimates of metabolism in this species. The correlations between enzymatic activities and buffering capacity (Fig. 1) or MO_2 (Fig. 2) were created using values for *O. deletron* fin muscle rather than mantle muscle. Furthermore, the specimens captured in previous studies were too few to permit accurate assessment of habitat depth for this species. Inclusion of the cranchiids and *O. deletron* does not significantly alter the results of any of the regressions using the depths in Table 1. Also excluded was the giant squid, *Architeuthis* sp. Minimum depth for this species is unknown: the only depth information comes from three juveniles captured at the surface (Roper and Young, 1972; Lu, 1986) and an adult tentacle that apparently broke off at 500 m (Robison, 1989).

Scaling

Citrate synthase activity generally declined with increasing body mass according to the equation $y = aW^b$, where a is a constant, W is wet weight, and b is the scaling coefficient. Scaling relationships for mantle muscle CS activity were significant in several cases (Fig. 6). The scaling relationships for *Histioteuthis heteropsis* ($y = 2.10x^{-0.25\pm0.14}$; $r^2 = 0.55$; $P < 0.01$; $n = 11$), *Chiroteuthis calyx* ($y = 4.20x^{-0.39\pm0.25}$; $r^2 = 0.76$; $P = 0.01$; $n = 7$), and *Japetella diaphana* ($y = 0.40x^{-0.29\pm0.15}$; $r^2 = 0.88$; $P = 0.004$; $n = 11$) were within the normal range found for aerobic metabolic scaling in a variety of animals (Schmidt-Nielsen, 1984). Significant scaling of CS activity in mantle muscle also exists for *Gonatus onyx* (Hunt and Seibel, 2000; Fig. 6) and *Vampyroteuthis infernalis* (Seibel *et al.*, 1998; Fig. 6). When data for the species were combined, there was significant negative scaling of mantle muscle CS activity for the family Cranchiidae as well ($y = 2.18x^{-0.29}$; $r^2 = 0.63$; $P = 0.04$; $n = 14$; Fig. 7). While significant positive scaling of fin muscle CS was previously found in *Vampyroteuthis infernalis* (Seibel *et al.*, 1998), no significant scaling relationships were found for fin muscle CS for any species in the present study.

Glycolytic enzymes of pelagic animals typically scale with a positive slope (Somero and Childress, 1980; Chil-

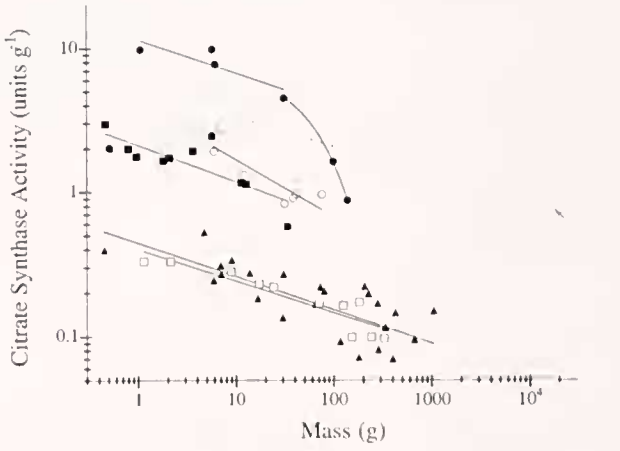

Figure 6. Citrate synthase activities for *Chiroteuthis calyx* (open circles; $y = 4.20x^{-0.39\pm0.25}$; $r^2 = 0.76$; $P = 0.01$; $n = 7$), *Histioteuthis heteropsis* (solid squares; $y = 2.10x^{-0.25\pm0.14}$; $r^2 = 0.55$; $P < 0.01$; $n = 11$), and *Japetella diaphana* (open squares; $y = 0.40x^{-0.29\pm0.15}$; $r^2 = 0.88$; $P = 0.004$; $n = 8$) as a function of body mass (g). Data are also shown for *Vampyroteuthis infernalis* (from Seibel *et al.*, 1998; closed triangles) and *Gonatus onyx* (from Hunt and Seibel, in press; closed circles). The two largest specimens of *G. onyx* were brooding egg masses and were in progressive stages of senescence. *Architeuthis* sp. (×) is shown at its estimated body mass of 20 kg. The dashed line represents an extrapolation to 10 g body mass using a scaling coefficient of −0.25.

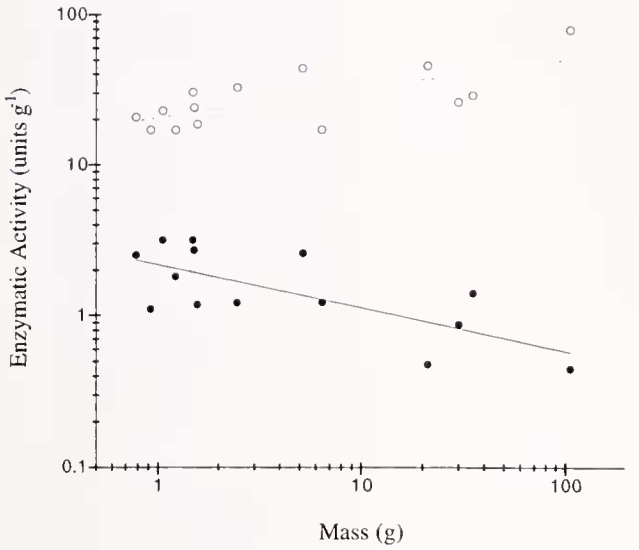

Figure 7. Activities of octopine dehydrogenase (open circles; $y = 20.6x^{0.20}$; $r^2 = 0.75$; $P = 0.002$; $n = 14$) and citrate synthase (closed circles; $y = 2.18x^{-0.29}$; $r^2 = 0.63$; $P = 0.04$; $n = 14$) in mantle muscle of individuals of cranchiid squid species (*Galiteuthis phylura, Cranchia scabra, Liocranchia valdivia, Sandilops* sp., *Taonius pavo*) as a function of body mass.

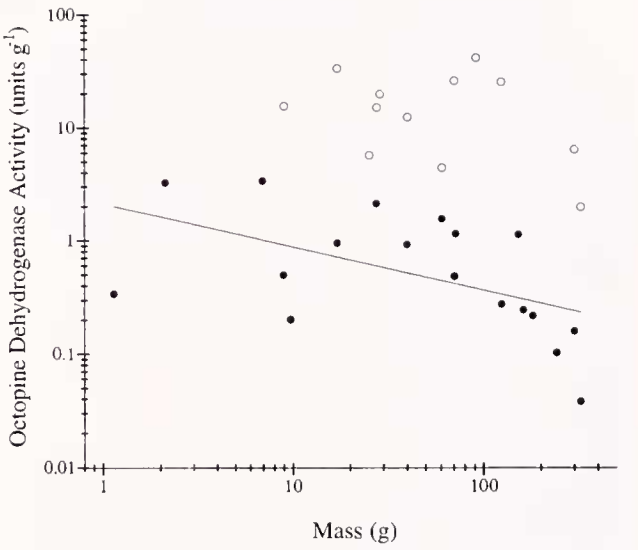

Figure 9. Octopine dehydrogenase activities in mantle (closed circles; $y = 2.12x^{-0.38\pm0.33}$; $r^2 = 0.28$; $P = 0.025$; $n = 18$) and arm (open circles) tissue as a function of body mass (g) for octopods within the family Bolitaenidae (*Japetella diaphana, J. heathi,* and *Eledonella pygmaea*).

dress and Somero, 1990; Seibel *et al.,* 1998). However, ODH activity in many cephalopod species or families in the present study either showed no relationship with body size or scaled negatively (Figs. 8, 9, 10). ODH scaled negatively for both mantle ($y = 5.03x^{-0.39\pm0.14}$; $r^2 = 0.62$; $P = 0.035$; $n = 7$) and fin ($y = 5.16x^{-0.33\pm0.06}$; $r^2 = 0.90$; $P = 0.014$; $n = 5$) muscle of *C. calyx* (Fig. 8). When data were combined, there was significant negative scaling of ODH activity in mantle muscle of bolitaenid species (*Japetella, Eledonella*) as well ($y = 2.12x^{-0.38\pm0.33}$; $r^2 = 0.28$; $P = 0.025$; $n = 18$; Fig. 9). No relationship existed between ODH activity in arm muscle and body mass for any species. ODH in mantle muscle for all cranchiid species combined scaled positively ($y = 20.6x^{0.20}$; $r^2 = 0.75$; $P = 0.002$; $n = 14$; Fig. 7). No significant scaling relationships existed for ODH activity of any histioteuthid species. When combined, however, ODH in fin muscle of *Histioteuthis heteropsis* and *H. hoylei* increased dramatically with increasing body mass ($y = 1.36x^{1.00\pm0.53}$; $r^2 = 0.62$; $P = 0.004$; $n = 8$; Fig. 10).

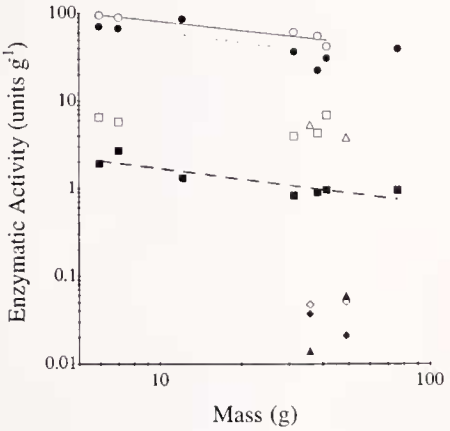

Figure 8. Activities of citrate synthase (squares) in fin (open symbols) and mantle (closed symbols; see Fig. 6) and octopine dehydrogenase (circles) in fin (open symbols; $y = 5.16x^{-0.33\pm0.06}$; $r^2 = 0.90$; $P = 0.014$; $n = 5$) and mantle (closed symbols; $y = 5.03x^{-0.39\pm0.14}$; $r^2 = 0.62$; $P = 0.035$; $n = 7$) of *Chiroteuthis calyx* as a function of body mass (g). For comparison, CS (triangles) and ODH (diamonds) in fin (open symbols) and mantle (closed symbols) for *Joubiniteuthis portieri* are also shown.

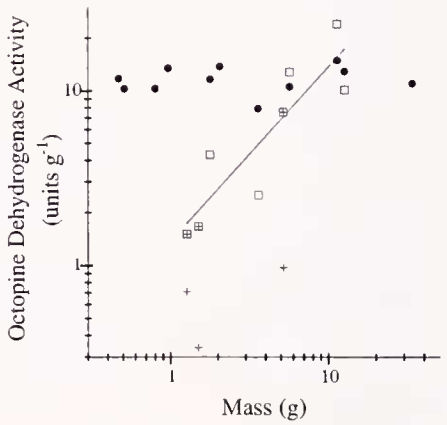

Figure 10. Octopine dehydrogenase activity of mantle muscle in *Histioteuthis heteropsis* (closed circles) and *H. hoylei* (plus signs) as a function of body mass (g). ODH activity in fin muscle (squares; $y = 1.36x^{1.00\pm0.53}$; $r^2 = 0.62$; $P = 0.004$; $n = 8$; Fig. 10) for *H. heteropsis* (open symbols) and *H. hoylei* (with plus signs) are also shown.

Critique of the Methods

Validation of enzyme measurements

In the present study we use V_{max} measurements of CS and ODH as indicators of maximal capacity for flux through aerobic and anaerobic metabolic pathways, respectively. Newsholme and Crabtree (1986) suggest two criteria that must be met in order to validate this approach. First, the enzyme must catalyze a nonequilibrium reaction. Newsholme and Crabtree (1986) suggest that there are a few master regulatory (nonequilibrium) enzymes in each pathway and that other near-equilibrium enzymes are in concentrations vastly exceeding the *in vivo* flux rates. Variation in the concentrations of near-equilibrium enzymes are, therefore, unrelated to environmental or ecological factors. CS is known to be an important regulatory enzyme that feeds carbon into the citric acid cycle (Hochachka *et al.*, 1975; Vetter, 1995). LDH and ODH, on the other hand, operate near equilibrium, and their concentrations are thought to be in excess of that required for forward flux through the glycolytic pathway. However, phylogenetic analysis has demonstrated that variations in LDH concentration do indeed have adaptive significance (Pierce and Crawford, 1997). Furthermore, it was recently demonstrated that the apparent excess concentrations of some near-equilibrium enzymes are closely matched to the requirements for flux through the pathway *in vivo* (Staples and Suarez, 1997). Octopine dehydrogenase is the only enzyme unique to anaerobic metabolism in cephalopods. Other supposed "flux-generating" enzymes in the glycolytic pathway participate in aerobic energy production as well. ODH is the terminal enzyme in energy-producing pathways when energy demand exceeds oxygen supply. Any energy expenditure beyond the capacity of aerobic pathways in the mitochondria must be covered by glycolysis, and pyruvate must be converted to octopine to maintain redox balance in the cytosol. The ODH-catalyzed step may, therefore, be considered rate-limiting.

The second requirement for useful measurement of V_{max} values is the experimental demonstration that they quantitatively indicate the *in vivo* capacity for flux through a pathway. This can be done by comparing the *in vitro* enzyme activity with the measured or calculated flux through the pathway, either directly by measurement of metabolite concentrations or indirectly from oxygen uptake measurements. In the case of anaerobic pathways, correlation with buffering capacity proves useful. In the present study, ODH activity in mantle tissue correlates well with buffering capacity (Fig. 1), whereas CS activity correlates with oxygen consumption (Fig. 2).

Temperature and pressure

Measurements of enzymatic activity in the present study were done under saturating substrate and co-factor concentrations, and are thus estimates of maximal capacity for metabolic flux (V_{max}) through the particular enzyme-catalyzed step in a pathway. V_{max} is a product of enzyme concentration ([*E*]) and catalytic efficiency (k_{cat}). For organisms living at similar environmental temperatures and hydrostatic pressures, k_{cat} values appear to be highly conserved, and V_{max} values are, therefore, essentially estimates of [*E*]. However, poikilotherms with low body temperature typically possess enzymes that are less rigid and thermally stable than those from warm-adapted species. Similarly, species from high-pressure environments also have more rigid enzymes that resist volume changes (Hochachka and Somero, 1984). Rigidity, required for enzyme stability, results in low catalytic efficiency. The deep sea is unique in that its inhabitants are exposed to both low temperature and high hydrostatic pressure. This trade-off results in some deep-living species having pressure-insensitive (rigid) enzymes that are only 60% as efficient (lower k_{cat}) as homologous enzymes from shallow-living species (Somero and Siebenaller, 1979). Although this difference may contribute to our observation of low enzymatic activity among deep-living cephalopod species, it is insufficient to explain the several orders of magnitude difference in enzymatic activity observed here between deep- and shallow-living cephalopod species. Rather, these differences in V_{max} result primarily from differences in [*E*] (Childress and Somero, 1979).

Destabilization of weak bonds by high temperatures can cause denaturation of proteins and thus inactivation of enzymes. The added rigidity of pressure-insensitive enzymes will increase their thermal stability to some extent. However, denaturation temperatures (*i.e.*, Arrhenius breakpoint temperatures) have been shown to correlate with species' habitat temperatures (Dahlhoff *et al.*, 1991). Midwater temperatures vary from roughly 3°C at 1500 m to 10° to 20°C at the surface, depending on region and season. The measurement temperature chosen here, 20°C, is significantly higher than the habitat temperature of most of the species studied. However, there is generally a large "buffer zone" between habitat temperature and denaturation temperature. Thus, the Arrhenius breakpoint temperature for most enzymes in most organisms is considerably greater than 20°C. Even the antarctic krill, *Euphausia superba,* which never sees temperatures above 2°C, has an optimum temperature of 40°C for citrate synthase (Vetter and Buchholz, 1998). Our own measurements in *Vampyroteuthis infernalis* and *Cirrothauma murrayi* as well as in antarctic pteropod molluscs and deep-sea benthic octopods revealed breakpoint temperatures greater than 35°C for ODH and LDH (Seibel, unpubl. data.). The measurement temperature chosen here is sufficiently high to allow greater detectability of enzymes that are in low concentrations. It is important to keep in mind, however, that *in situ* temperature differences between species will lead to a greater difference in metabolic rate

than indicated by the enzymatic activities measured here (Childress and Somero, 1979).

Phylogeny

The analyses presented here generally assume, for statistical purposes, the independence of species as individual data points. Some authors (Harvey and Pagel, 1991; Felsenstein, 1985) have warned, however, that physiological traits of species reflect, to varying degrees, both their historical environments and their phylogenetic affinities. Failure to consider phylogenetic relationships for statistical analyses may overestimate the degrees of freedom for regressions. An "analysis of higher nodes" (Harvey and Pagel, 1991) presents one possible solution to this problem. This analysis partitions the total variation among species into components representing each of the nested levels in a taxonomy (*i.e.*, the variation between species within a genus, genera within a family, families within an order, etc.). Such an analysis on the present data set reveals that the majority of the variation lies between families within an order, and thus families are the appropriate taxon for the most independent comparisons (Seibel, 1998). However, with few exceptions, there is only a single representative from a given family within each region studied (California or Hawaii). Therefore, our degrees of freedom for each regional analysis are not greatly exaggerated. Reanalysis using mean enzymatic activities for each family does not significantly change any of our regressions or conclusions (Seibel, 1998). For midwater cephalopods, phylogenetic trees based on sequences of the cytochrome *c* oxidase I gene have recently become available (Carlini and Graves, 1999). Preliminary analysis of the present data set using phylogenetic independent contrasts (Felsenstein, 1985) based on these phylogenetic trees (Carlini and Graves, 1999) appear to support our findings (Carlini and Seibel, unpubl. data). Childress (1995) argued that low metabolic rates among midwater organisms are probably not a phylogenetic artifact for two reasons. First, there is considerable divergence of closely related groups as a function of habitat depth, and second, there is strong evidence of convergence between distantly related groups (phyla) living at similar depths.

Discussion

Depth

Citrate synthase (CS) activity and octopine dehydrogenase (ODH) activity in mantle muscle decline significantly with increasing minimum depth of occurrence (Figs. 3, 4). This is in agreement with the visual interactions hypothesis, which states that deeper living animals will have lower metabolic rates than their shallower living counterparts as a result of relaxed selection for strong locomotory abilities for predator-prey interactions in the light-limited deep sea (Childress, 1995). This hypothesis is supported by the presence of a decline among visually orienting pelagic animals (fishes, crustaceans, and cephalopods; Childress, 1995; Seibel *et al.*, 1997), and the lack of a decline among nonvisual pelagic organisms, such as medusae and worms (Thuesen and Childress, 1993a, b, 1994). It is further supported by the lack of a decline among benthic organisms that have greater opportunities for refuge and crypsis (Childress *et al.*, 1990; Seibel and Childress, 2000). In shallow benthic environments, visually cued predation pressure selects for chromatophore patterns and other cryptic displays rather than for powerful propulsive muscles that allow escape from predators. Similarly, in the deep sea, the limited visual interaction that occurs between cephalopods and their predators has resulted in cryptic behaviors such as bioluminescence. Although low metabolic rates are certainly advantageous for life in regions of low oxygen or low food availability, regional comparisons have shown that metabolic rates are not specific adaptations to these factors (Childress, 1995; Seibel *et al.*, 1997; Childress and Seibel, 1998; Seibel *et al.*, 1999). The finding that enzymatic activities are similar for cephalopod species from California and Hawaii, regions that differ dramatically in their oxygen and biomass profiles, supports this assertion.

Further evidence of reduced metabolism and locomotory capacity among deep-sea cephalopods was provided by enzymatic activities in branchial heart muscle. We observed a significant decline in both CS and ODH activity in branchial heart tissue of pelagic cephalopods (Fig. 5). This makes sense, given a reduction in overall metabolic and locomotory expenditure in the deep sea. In fishes, however, this reduction is apparently accomplished only by a reduction in heart size (Childress and Somero, 1979). Ratios of CS to ODH are higher in branchial heart muscle than in locomotory muscles of cephalopods, reflecting the typical aerobic poise of heart metabolism (Dreidzic *et al.*, 1990). The relatively high activities of LDH at all depths in branchial heart muscle may be poised to reconvert lactate, produced in low concentration by the large locomotory muscles, to pyruvate as discussed by Storey (1977). LDH in both mantle and heart muscle is similar at all depths. Octopine, the product of arginine and pyruvate, is typically metabolized in the muscle tissue of cephalopods (Pörtner, 1994). This allows the resynthesis of arginine-phosphate, a high-energy buffer equivalent of creatine phosphate in vertebrates, in active muscles. ODH in heart tissue of cephalopods is probably related to increased work during and after burst locomotion.

Locomotion

Oxygen consumption in pelagic cephalopods declines with increasing habitat depth more sharply (higher slope, b) than in either fishes or crustaceans (Seibel *et al.*, 1997). The

same trend appears to be true for CS ($b = -1.23$) and ODH ($b = -1.54$) activity in cephalopod mantle muscle (Figs. 3, 4) compared to malate dehydrogenase ($b = -0.68$) or LDH ($b = -0.69$) in fish white muscle (Childress and Somero, 1979). Seibel *et al.* (1997) suggested that the relative steepness of these relationships is a result of differences in locomotory efficiency between deep and shallow-living cephalopods. Because fin swimming is inherently more efficient than jet propulsion (Hoar *et al.*, 1994), and because high speeds are not a priority in the deep sea, much of the observed reduction in energy usage among deeper living cephalopod species may result from their use of fins as the primary means of propulsion (Wells, 1994; Seibel *et al.*, 1997).

The decline in mantle muscle CS and ODH activity and the lack of a decline in fin muscle CS and ODH with depth support the contention that there is a relative increase in the use of fins for propulsion among deeper living animals compared to the use of mantle muscle. The lack of a decline in arm muscle CS and ODH suggests either that deeper living species are utilizing arms for medusoid swimming, or that species at all depths utilize arms for activities, such as defense or prey capture, that require similar metabolic potential regardless of habitat depth. Medusoid swimming has been observed previously for cirrate octopods (Vecchione and Roper, 1991; Vecchione and Young, 1997; Villanueva *et al.*, 1997) and some histioteuthid squids (Voss *et al.*, 1998). These possibilities are explored further below.

The patterns of enzymatic activity with habitat depth support the contention that deeper living species generally have lower metabolic rates and increased efficiency of locomotion due, in part, to a greater relative use of fins for both routine and burst swimming. However, the enzymatic activities presented here and elsewhere (Seibel *et al.*, 1998; Hunt and Seibel, 2000) vary considerably within a species as a function of body mass or ontogeny. Consideration of intraspecific scaling relationships reveals a much clearer picture of the relative distribution of enzymes in locomotory tissues of midwater cephalopods.

Scaling and ontogenetic vertical migration

Aerobic metabolic enzymatic activities (CS) in *Histioteuthis heteropsis, Chiroteuthis calyx, Japetella diaphana, Vampyroteuthis infernalis* (Seibel *et al.*, 1998), and *Gonatus onyx* (Hunt and Seibel, 2000) decreased significantly with increasing body mass (Fig. 6). A similar relationship was observed for species within the family Cranchiidae (Fig. 7). Mass-specific aerobic metabolism typically decreases with increasing body mass (Schmidt-Nielsen, 1984). The evolutionary cause of these relationships is not well understood, but is believed to derive from increased opportunities for energy savings in larger animals as a result of reduced costs for such geometrically controlled (*i.e.*, surface:volume) processes as thermoregulation in mammals or ion regulation in fishes (Childress and Somero, 1990).

Childress and Somero (1990) also discussed scaling of glycolytic enzymes in fishes and concluded that the positive scaling coefficients for most locomotory muscles are an adaptation for size-independent acceleration used during predator-prey interactions. Larger animals are required to accelerate proportionally faster than small animals to escape predators or capture prey. This is dependent on the scaling of predator-prey detection distances. The positive scaling of LDH activity observed for *Corphaenoides armatus* (Siebenaller *et al.*, 1982), a permanently deep-living fish, supports this hypothesis, as does the positive scaling of ODH activity in *Vampyroteuthis infernalis* mantle, fin, and arm tissue (Seibel *et al.*, 1998). Because *C. Armatus* and *V. infernalis* live at constant depths (constant light levels) through ontogeny, detection distance is determined primarily by body size.

The absence of significant scaling relationships or the presence of negative scaling relationships for enzymes of anaerobic metabolic pathways among many midwater cephalopods (Figs. 8, 9, 10) may reflect the ontogenetic vertical migrations undertaken by these species whereby successive developmental stages occupy progressively deeper depths (Roper and Young, 1975). Shallow-living juveniles of such species experience greater visibility, but detection distances are small due to their small size. Deep-living adults experience reduced visibility in the poorly lit deep sea, so that detection distances remain small even though the animals are larger. This trade-off may negate the positive scaling of anaerobic metabolism typically observed in pelagic animals.

The negative scaling of ODH activity in *Gonatus onyx* (Hunt and Seibel, 2000) supports this hypothesis. *G. onyx* undergoes an ontogenetic migration to great depths where spawning and egg-brooding take place (Hunt and Seibel, 2000; Seibel *et al.*, 2000; see also Fig. 6). It is further supported by negative scaling of ODH in *Chiroteuthis calyx* (Fig. 8) and in mantle muscle of bolitaenid octopods (Fig. 9), as well as by the lack of scaling relationship for ODH in *Histioteuthis heteropsis* mantle muscle (Fig. 10). All of these species undergo ontogenetic migrations to deep water (Roper and Young, 1975).

ODH in mantle muscle within the family Cranchiidae scales positively (Fig. 7). Although members of this family undergo ontogenetic vertical migrations, their use of transparency for crypsis reduces their predator/prey detection distances regardless of habitat depth (Seibel *et al.*, 1997; Johnsen and Widder, 1998). Detection distance depends only on size for these species.

Chiroteuthid clade

When body size is factored out, enzymatic activity in fin muscle and mantle muscle can be compared. From Figure 8

it can be seen that fin muscle CS and ODH activity is much higher than mantle muscle activity at all body sizes for *Chiroteuthis calyx*, *C. imperator*, *Joubiniteuthis portieri*, and *Mastigoteuthis famelica*, all members of the chiroteuthid clade (Young, 1991; Carlini and Graves, 1999), are also apparently strong fin swimmers. In fact, *J. portieri* has virtually no enzymatic activity in mantle muscle, but the CS activities in its fin muscle are similar to those in related midwater squids. In a shipboard aquarium, *Joubiniteuthis portieri* swam vigorously with constant fin motion and only a few mantle contractions beyond those used for respiration (Seibel, pers. obs.). The enzymatic activities of *M. famelica* do not unambiguously support fin swimming. Although CS activity is higher in fin than mantle muscle, the reverse is true of ODH activity. However, this species has been observed from submersibles to be swimming almost exclusively using the fins (Roper and Vecchione, 1997). Furthermore, the regions of the brain associated with fin swimming are extremely well-developed in mastigoteuthid species (Dilly *et al.*, 1977; Young, 1977), and the fins themselves are quite large. The low ODH activity in fin relative to mantle muscle in *M. famelica* may reflect the small size (4–7 g) of these specimens, because jet propulsion may be more efficient than fin swimming at small sizes (O'Dor and Webber, 1986). Scaling relationships are not known for this species.

Histioteuthidae

ODH activity in fin muscle of *Histioteuthis heteropsis* and *H. hoylei*, combined, scales with a large positive slope (Fig. 10). Mantle muscle ODH of *H. heteropsis* is independent of body mass (Fig. 10). The significance of this finding is that fin muscle in deeper living adult *H. heteropsis* has higher ODH activity than mantle muscle, which suggests an ontogenetic gait-transition whereby the use of fins for burst swimming becomes relatively more important through ontogeny. This transition coincides with an ontogenetic descent to deeper, darker water. Jet propulsion may actually be more efficient than fin swimming at small sizes (O'Dor and Webber, 1986). An ontogenetic gait-transition was reported for *Vampyroteuthis infernalis* (Seibel *et al.*, 1998) as well. However, in that case, the transition from jet propulsion to fin swimming occurs as a distinct metamorphosis, rather than gradually through ontogeny.

Despite similar fin muscle ODH activities and metabolic rates (Seibel *et al.*, 1997), *Histioteuthis heteropsis* and *H. hoylei* have drastically different mantle muscle ODH activities. It appears that fin muscle enzymatic activities are always considerably higher than mantle muscle activities in *H. hoylei*. The mantle is also relatively longer in *H. heteropsis* than in *H. hoylei* (Voss *et al.*, 1998). *Histioteuthis hoylei* may rely on fins for swimming at all sizes and depths. Some histioteuthids have been observed swimming with their arms, like medusae and cirrate octopods (Voss *et al.*, 1998; see below). Enzymatic activities do not support this possibility for *H. hoylei*, however.

Bolitaenidae

Enzymatic activity is much higher in arm muscle than in mantle muscle of bolitaenid octopods (*Japetella diaphana*, *J. heathi*, *Eledonella pygmaea*; Fig. 9). A similar pattern was observed for the cirrate octopod *Opisthoteuthis californiana* (Seibel *et al.*, 1998). This may reflect the use of arms for medusoid swimming, as has been observed for some cirrate octopods (Roper and Brundage, 1972; Vecchione and Roper, 1991; Vecchione and Young, 1997). However, limited shipboard observations on swimming behavior in *Japetella heathi* did not reveal medusoid swimming. Rather, mantle muscle was predominantly used for jet propulsion. Furthermore, *J. heathi* appeared to expel water through the lateral mantle "inlets" in addition to the funnel. A similar "triple jet" arrangement was described for the epipelagic octopod *Ocythoe tuberculata* (Packard and Wurtz, 1994). At extremely low speeds, jet propulsion is apparently not so costly (Vogel, 1994). A triple jet arrangement allows bolitaenids to process larger amounts of water, resulting in efficient low-speed swimming. The high enzymatic activity in arm tissue may be used in prey capture or defense.

Architeuthidae

The giant squid, *Architeuthis* sp. reaches lengths of over 20 m. Despite its enormous size, no biologist has ever seen one alive in its natural habitat. The elusiveness of this species has spurred considerable myth regarding its predatory abilities (Ellis, 1998). However, many scientists now agree that *Architeuthis* is a sit-and-wait predator with little swimming ability (Bidder, 1970). This belief is based primarily on the observation that *Architeuthis* accumulates ammonium in its muscle tissues, presumably for buoyancy (Voight *et al.*, 1994; see below). The present measurements provide the first estimates of metabolism and locomotory ability for this poorly known genus. The mantle muscle CS activity, according to the relationship in Figure 2, predicts an oxygen consumption rate of 0.50 μmole O_2 $g^{-1}h^{-1}$, which is similar to that of most ammoniacally buoyant squids (Seibel *et al.*, 1997). However, normalizing this value allometrically to 10 g body mass for comparison with other species (assuming a scaling coefficient of -0.25; Fig. 6) gives a value of 3.34 μmole O_2 $g^{-1}h^{-1}$. This value is closer to muscular squids, such as the enoploteuthids and gonatids, than to the ammoniacal squids with which it is commonly compared (Fig. 6; Table 1).

The capacity for burst swimming appears to be quite low. Octopine dehydrogenase activity (7.22 units g^{-1}) is lower than that found here for some ammoniacal species (Table 1). *Architeuthis* is believed to undergo an ontogenetic migra-

tion. Juveniles have been found at the surface (Roper and Young, 1972; Lu, 1986), whereas adults are believed to reside in midwater (Robison, 1989). If *Architeuthis* does indeed undergo an ontogenetic descent, then we might assume that juveniles have much greater anaerobic capacity than adults (see Figs. 8, 9; and Hunt and Seibel, 2000).

O'Dor (1988) suggested, on the basis of scaling of migratory ranges in smaller squid, that *Architeuthis* could, if it wanted to, circle the globe in under 80 days. He suggested that the apparently weaker muscle tissue of *Architeuthis* relative to shallow-living squids is not necessarily evidence against strong swimming ability *per se*. He suggested, rather, that burst swimming may be limited and that perhaps *Architeuthis* requires only "cruising muscle" (O'Dor, 1988). This hypothesis is consistent with the relatively high aerobic, but low anaerobic, capacity measured here.

The present specimen was kept on ice or in a refrigerated cold room for at least 3 days prior to dissection. It is not known how long it had been dead at the surface of the ocean before collection or to what extent the enzymes may have degraded. We view the present measurements as conservative estimates.

Buoyancy

Many midwater cephalopods possess some means of achieving neutral buoyancy, whereas most epipelagic squids are negatively buoyant and rely on constant swimming for lift. Some midwater squids accumulate high quantities of ammonium ions that are lighter than seawater and thus provide a buoyant force (Denton and Gilpin-Brown, 1973; Clarke *et al.*, 1979; Voight *et al.*, 1994). The difference in metabolic potential between shallow- and deep-living species may, therefore, be partially accounted for by differing energy requirements for support in the water column as a result of interspecific differences in density.

However, strong locomotion requires a high protein content that precludes neutral buoyancy. Therefore, relaxed requirements for strong locomotory abilities allow the exploitation of buoyancy mechanisms. The need for strong swimming determines the practicality of buoyancy (Childress and Nygaard, 1974; Seibel *et al.*, 1997). Some of the variation in enzymatic activity at a given habitat depth certainly reflects differences in the type of buoyancy mechanism employed or in the relative distribution of buoyancy-related compounds within the body. For example, the cranchid and chiroteuthid squids have very high mantle muscle ODH activities relative to histioteuthids despite having similar oxygen consumption rates (Seibel *et al.*, 1997). This may be, in part, due to differing distributions of ammonium in the bodies of these groups. The cranchiids store ammonium in a separate coelomic compartment and have none in the mantle tissue. Chiroteuthids store large quantities of ammonium in two enlarged arms (as adults) and have relatively little in mantle muscle. Histioteuthids, on the other hand, store the majority of their ammonium in mantle tissue (Denton and Gilpin-Brown, 1973; Clarke *et al.*, 1979; Voight *et al.*, 1994). Despite these differences, we do not believe that the overall patterns of enzymatic activity with depth or body mass are specifically related to buoyancy.

Light-limitation on predator-prey interactions

The ecological and evolutionary implications of low levels of ambient light in the deep sea have long been recognized (see reviews in Herring *et al.*, 1990). By reducing predator-prey detection distances, low light levels in the deep sea influence behavior (Hunt and Seibel, 2000), vertical distribution (Young *et al.*, 1980), morphology, reproduction (Seibel *et al.*, 2000), and metabolism (Childress, 1995; Seibel *et al.*, 1997). Relaxed selection for strong locomotory abilities in the deep sea allows the adoption of energy-saving buoyancy mechanisms (Childress and Nygaard, 1974). Similarly, we argue here that relaxed requirements for locomotory ability have allowed the evolution of efficient means of locomotion by deep-sea cephalopods. Enzymatic activities from the present study generally appear to correlate well with locomotory behaviors previously hypothesized on the basis of morphology (Bidder, 1970; Clarke, 1988), angular acceleration receptors (Young, 1989), brain structure (Young, 1977), or direct observations from submersibles (Hunt, 1996). In the deep sea, where high speeds are not strongly selected for, use of fins or arms for locomotion reduces the cost of transport. We point out only a general trend toward more efficient means of locomotion in the deep sea: the tremendous variation in swimming behaviors and morphologies among species contributes much of the variation to our data set.

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